

Claims:

1 1. A single-stranded-oligonucleotide DNA primer for amplification of a target
2 DNA sequence capable of use in a multiplex polymerase chain reaction (PCR), said primer
3 having the structure 5'-XY-3', wherein

4 a) X comprises a sequence that does not hybridize to said target sequence;

5 b) the melting temperature of a hybrid between X and its complement in the
6 absence of other sequences is greater than about 60°C; and

7 c) Y comprises a sequence contained within or flanking said target sequence or
8 its complement.

1 2. The primer of claim 1, wherein X comprises the sequence
2 5'-GCGGTCCCAAAGGGTCAGT-3'.

1 3. The primer of claim 1, wherein X and Y each comprise from 17 to 20
2 bases.

1 4. The primer of claim 1, wherein the melting temperature of a hybrid formed
2 between said primer and its complement in a solution of 0.5M NaCl is at least 72°C.

1 5. An oligonucleotide DNA primer for amplification of a target DNA
2 sequence, wherein said primer consists of the sequence 5'-GCGGTCCCAAAGGGTCAGT[Y]-

3 3', wherein Y comprises a sequence contained within or flanking said target sequence or its
4 complement.

1 6. A method for simultaneous amplification of multiple DNA target sequences
2 present in a DNA sample, which comprises:

3 a) contacting said DNA sample in a single reaction mixture with a multiplicity of
4 paired oligonucleotide primers having the structure 5'-XY-3', wherein

5 (i) X comprises the sequence

6 5'-GCGGTCCCAAAAGGGTCAGT-3', and

7 (ii) Y comprises a sequence contained within or
8 flanking said target sequence or its
9 complement; and

10 b) performing multiple cycles of melting, reannealing, and DNA synthesis.

1 7. A method for detecting multiple defined target DNA sequences in a DNA
2 sample, which comprises the steps of:

3 a) contacting said DNA sample in a single reaction mixture with a multiplicity of
4 oligonucleotide pairs, each of said pairs consisting of a first and second oligonucleotide primer,
5 wherein

6 (i) said first primer of each pair has the structure 5'-XY-3', wherein X
7 comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' and Y comprises a sequence
8 contained within the target sequence or its complement, and

9 (ii) said second primer of each pair has the structure 5'-XY-3', wherein
10 X comprises the sequence 5'-GCGGTCCCAAAGGGTCAGT-3', and Y comprises a sequence
11 flanking the target sequence or its complement;

12 b) performing multiple cycles of melting, re-annealing, and DNA synthesis to
13 form amplification products of DNA samples primed with said oligonucleotides; and

14 c) detecting the amplification products.

1 8. The method of claim 7 wherein detection of an amplification product
2 indicates the presence of the target sequence in the DNA sample.

1 9. The method of claim 7 wherein said detecting step comprises gel
2 electrophoresis.

1 10. A method for high-throughput genetic screening to simultaneously detect
2 the presence of multiple defined target DNA sequences in DNA samples obtained from a
3 multiplicity of individuals, said method comprising the steps of:

4 a) providing a sample of DNA from each of said individuals;

5 b) simultaneously contacting each of said DNA samples obtained in a) with a
6 multiplicity of oligonucleotide pairs, each of said pairs consisting of a first and second
7 oligonucleotide primer, wherein

8 (i) said first primer of each pair has the structure 5'-XY-3', wherein X
9 comprises the sequence 5'-GCGGTCCCAAAGGGTCAGT-3' and Y comprises a sequence
10 contained within the target sequence or its complement, and

11 (ii) said second primer of each pair has the structure 5'-XY-3', wherein
12 X comprises the sequence 5'-GCGGTCCCAAAGGGTCAGT-3', and Y comprises a
13 sequence flanking the target sequence or its complement;

14 b) performing multiple cycles of melting, re-annealing, and DNA synthesis to
15 form amplification products; and

16 c) detecting the amplification products.

1 Sub 11. The method of claim 10 wherein detection of an amplification product
2 DS indicates the presence of the target sequence in the DNA sample.

1 B 12. The method of claim 10 wherein said detecting step comprises gel
2 electrophoresis